Workshop J
Antibody-mediated Autoimmunity

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J.1 Rheumatoid arthritis (RA): Disease specific autoantibodies

S. Bläss[1], C. H. Specker[2], H.-J. Lakomek[3], and M. Schwochau[1]

66 % of RA patients (n = 167) carry autoantibodies detecting a possibly ubiquitously expressed 68 kD antigen that is present in total protein preparations of all human tissues investigated (synovium, lymphocytes, sperma, liver, and HeLa S3 cells). The antibodies do not occur in healthy controls (n = 55). They are present in only 6 % of patients with other rheumatic diseases (n = 97). Taking into account that almost all patients of the latter control group with anti-68 kD antibodies showed clinical and radiological overlap to RA, it turns out that the specificity of these particular antibodies for RA is nearly 100 %. Furthermore, there is a strong correlation between the presence of the antibody and a more severe course of the disease. These findings will not only allow for a diagnosis of RA superior to that based on the rheumatoid factor; in addition, the analysis of the gene coding for the 68 kD antigen will provide means for the investigation of the pathomechanisms resulting in RA. The 68 kD antigen does not appear to be a stress protein or the autoantigen encountered in type I diabetes. The isoelectric point of 5.1 suggestes that it is also not one of the RNP proteins.

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J.2 Characteristics of an anti-CD4 antibody useful in monitoring and treatment of chronic autoimmune arthritis

F. Emmrich[1], W. Becker[3], G. Burmester[2], W. Lüke[4], G. Horneff[2], W. Seiler[5], A. Potochnik[1], and J. Kalden[2]

Monoclonal antibodies (mAb) to CD4 inhibit the function of $CD4^+$ T cells *in vitro* and have been used for treatment of autoimmune diseases in several animal models. We have prepared an anti-CD4 mAb (MAX.16H5) that binds with high affinity to human CD4 ($K_D = 5 \times 10^{-10}$M). MAX.16H5 modulates CD4 more efficiently than other anti-CD4 antibodies used in therapeutical trials (OKT4, T151) and is superior with respect to inhibition of HIV-infection and gp120 (HIV) binding as compared to OKT4A and Leu 3a. When used in

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patients, MAX.16H5 elicits a marginal anti-mouse-immunoglobulin response including anti-idiotypic (anti-Id) antibodies. Polyclonal as well as monclonal anti-Id antibodies were prepared to define the major epitopes of the anti-Id response. MAX.16H5 depletes $CD4^+$ cells from circulation and has been successfully used for treatment of human rheumatoid arthritis with improval in clinical and laboratory parameters. It accumulates at the site of inflamed joints thus permitting imaging with 99mTc-labelled antibody. Affected digital joints were detected earlier by virtue of helper T cell imaging than by conventional bone scans.

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J.3 Characterization and biological functions of IgG2a-reactive autoantibodies isolated from virus infected BALB/c mice

G. Fazekas[1], E. Rajnavölgyi[1], I. Kurucz[2], G. Laszlo[1], and J. Gergely[1]

IgG2a-reactive autoantibody producing hybridomas have been isolated from the spleen of influenza virus-infected BALB/c mice. These IgM, IgG and IgA type monoclonal antibodies (mAb) do not cross react with a panel of self and non-self structures and show strong isotype or iso-allotype specificity. The common functional property of these mAbs is their preferential and high affinity binding to complexed or aggregated IgG2a compared to native soluble IgG2a. The IgM and IgA type mAbs do not bind to cell surface bound IgG2a while the IgG2b antibody recognizes membrane IgG2a as well. IgM and IgG type mAbs are not, while the IgA type mAb is able to inhibit the binding of complexed IgG2a to the high affinity Fc receptor (FcRI) of the human monocytic cell line U937. This activity is in good correlation with its iso-allotype specificity determined by the amino acid residue Ala at position 305 of the C_H2 domain. The restricted specificity and functional properties of these rheumatoid factor (RF) like autoantibodies raise the possibility of their regulatory role in the ongoing immune response. Results of our *in vivo* experiments demonstrate that passive administration of the IgM-type IgG2a-specific autoantibodies to influenza virus-infected or oxazolone-sensitized mice result in a long term suppression of the secondary IgG2a antibody response while other isotypes are not affected. These results suggest the isotype-specific regulatory function of certain RF-type autoantibodies depending on their fine specificity.

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J.4 Human rheumatoid factor light chains may be encoded by a V_κ IV light chain gene

A. Gause[1], R. Küppers[1], E. Albani[1], E. Genth[2], and R. Mierau[2]

As nearly all known light chains of IgM rheumatoid factors (RF) belong to the variable region V_κ IIIb (V_κ IIIb) subgroup, we sequenced the light chain from the IgA RF producing hybridoma P61B27 derived from a patient with seropositive rheumatoid arthritis. The sequencing was done from cDNA clones established by specific priming of messenger RNA with a human constant region primer. The sequence of a full length light chain V region clone revealed 91 % homology

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to the V_κ IV subgroup which is encoded by a single germline gene. Southern blot hybridization with the corresponding V_κ IV leader probe (KLOBECK et al. 1985, Nucl. Acids Res. 13: 6515) revealed that V_κ IV was rearranged in the hybridoma P61B27 only and not in the fusion partner K6H6/B5. Sequence differences (18 replacement and 8 silent mutations in 104 codons) to the known V_κ IV germline gene may be due to somatic mutation or polymorphism in the human population. Interestingly, 4 of 18 replacement mutations (position 4 and 12 in the framwork 1 and position 91 and 94 in the hypervariable region 3) lead to identical amino acids as in the RF prototype light chain V_κ IIIb. Therefore these regions may be important for RF activity and our observation may argue for a selection of certain amino acids for RF function. The heavy chain V region gene is presently being investigated.

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J.5 Adverse immune reactions to a gold(I) drug are directed to gold(III), not gold(I)

M. KUBICKA-MURANYI, D. SCHUHMANN, J. GÜNTHER, P. KIND, and E. GLEICHMANN

Compounds containing gold in the gold(I) oxidative state such as gold sodium thiomalate (GST), are effective in the treatment of rheumatoid arthritis. However, in up to 1/3 of the patients adverse immune reactions necessitate discontinuation of gold therapy. In the genetically susceptible A.SW mouse strain we induced increased levels of total IgE, IgG, antinucleolar autoantibodies, and glomerular IgG deposits by repeated i.m. injections of GST. However, to our surprise the gold(I) drug GST failed to elicit reactions in the direct popliteal lymphnode assay (PLNA), which is a sensitive method for detecting T cell-dependent reactions to small chemicals. Gold(III) compounds on the other hand elicited strong and specific PLN reactions. To explain the paradox of adverse immunological side-effects seen after repeated injections of GST on the one hand and the lack of response to GST in the direct PLNA on the other hand we postulated that *in vivo* the non-immunogenic gold(I) of GST is gradually oxidized to the immunogenic gold(III). This indeed seems to be the case: By using the adoptive transfer PLNA, splenic T cells obtained from A.SW mice, which had received weekly i.m. injections of GST for 12 weeks and were autoimmune, were shown to be sensitized to gold(III), but not to gold(I) or ST. The anatomical site where biooxidation of gold(I) takes place may be in the peroxisomes/lysosomes («aurosomes») of macrophages.

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J.6 B-cell autoregulation *in vitro*

A. MAGYAR

It had recently been described that the supernatant of LPS-activated B cells suppresses the proliferation of mitogen-activated B lymphocytes *in vitro*. The role of autoantibodies was

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suggested during this process. We could confirm and extend this observation. We studied the mechanism of this inhibition with flow cytometrical analysis. The results of measuring DNA contents of B cells treated with the supernatants show that there is no passing from the $G_0 + G_1$ into the S and $G_2 + M$ phases of the cell cycle in cultures. The simultaneous detection of DNA and RNA content proved that in the presence of the B-cell supernatant the LPS-treated B cells can't pass the G_1 phase. The vital staining excluded the possibility that the inhibitory effect is the result of increased cell death. Parallel with flow cytometry we also measured the polyclonal antibody production in these B-cell cultures by determining the total IgM and IgG content of the culture fluids. Finally we demonstrated the changes in the level of some type of autoantibodies.

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J.7 Ankylosing spondylitis (AS)-specific autoantibodies: Characterization of the respective antigens

M. Plomann[1], St. Bläss[1], Ch. Specker[2], H.-J. Lakomek[3], and M. Schwochau[1]

In the sera of AS patients we identified autoantibodies specific for this systemic inflammatory disease. These antibodies are present in patients with and without the HLA-B27 haplotype in an equal proportion. These specific autoantibodies can also be detected in other «seronegative» spondylarthropathies showing the clinical and radiological changes typical for AS. These autoantibodies, however, are not detectable in sera of apparently healthy controls or RA patients, respectively.

A most prominent reaction of the AS specific antibodies can be seen with a 36 kD antigen in Western blots of total protein preparations from insect tissue known to be reactive with anti-AS antibodies. The isoelectric point of this insect antigen is 9.0. It is not heat inducible. Antibodies affinity-purified from the 36 kD insect antigen detect a 69 kD human antigen in Western blots of total protein preparations from lymphocytes, synovium and HeLa cells.

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J.8 *In vitro* and *in vivo* characteristics of a chimeric human-mouse monoclonal CD4 antibody

C. Reiter[1], H. Feucht[2], E. P. Rieber[1], M. Dalesandro[3], J. Grayeb[3], P. Daddona[3], G. Riethmüller[1]

Monoclonal CD4 antibodies (CD4 mAb) of mouse origin have been successfully used for treatment of kidney allograft rejection and rheumatoid arthritis (1). The mode of action and parameters predictive for clinical efficiency of CD4 mAb are still unknown. By means of genetic engineering the constant regions of the mouse IgG2a,κ CD4 mAb M-T412 were substituted for the constant parts of human IgG1,κ to minimize antigenicity and to improve

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recruitment of human effector mechanisms. Murine M-T412 and chimeric M-T412 (chimM-T412) had the same affinity and epitope specificity. However, chimM-T412 was distinctly more effective in blocking lymphocyte proliferation on various stimuli. *In vivo* data indicate a short serum half live in the order of a few hours for both antibodies. Marked immunosuppressive capacity of chimM-T412 was demonstrated in the treatment of acute corticosteroid resistant renal allograft rejection.

1. C. Herzog, C. Walker, W. Müller et al.: Anti-CD4 antibody treatment of patients with Rheumatoid Arthritis: I. Effects on Clinical course and circulating T cells. Autoimmunity (1989) 2:627–642.

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J.9 Complement activating and ADCC mediating properties of anti-IL-2R monoclonal antibodies

O. Rentrop, H. Hock, H.-D. Volk, and T. Diamantstein

Monoclonal antibodies (mAb) of different isotypes, directed against the light chain of the interleukin-2 receptor (IL-2Rp55), have been used in the past to combat autoimmune diseases and malignancies in humans with variable success. We have isolated switch variants (IgG1 → IgG2b → IgG2a) of the mouse-α-IL-2Rp55-mAbs AHT107 and AHT54 and examined their capability to activate natural effector mechanisms *in vitro* (complement dependent cytotoxicity [CDC], activation of C3 fragments and antibody dependent cell mediated cytotoxicity [ADCC]) in order to obtain indications to their potential efficiency *in vivo*. In all *in vitro* studies, human ConA-stimulated T cells and the IL-2Rp55$^+$ cell line L540 were used as targets. Since AHT107 and AHT54 recognize different epitopes on the IL-2Rp55 they were also tested in combinations. Only combinations of AHT107 and AHT54, but none of the different isotypes alone were able to activate human complement with subsequent deposition of C3 fragments on the target cell surface. The combination of different isotypes in all possible permutations showed, that the amount of C3-fragment deposition was isotype-dependent (IgG2a ≥ IgG2b >> IgG1). Yet, none of the different isotypes, neither alone nor in combinations were able to lyse the target cells, when human serum was used as source of complement. Using rabbit complement, IgG2b- and IgG2a-, but not IgG1-mAbs could mediate CDC. In ADCC, we also compared different rat-α-IL-2Rp55-mAbs (IgG2b and IgG2a) with the mouse switch variants. Most effective was the rat-IgG2b, the only mAb which was capable to mediate ADCC with human PBMC. Using monocyte-enriched adherent cells, pairs of mouse-IgG2a also showed a modest activity.

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J.10 Detection of autoantibodies against exocrine pancreas by double immunodiffusion testing

Wiebke Rögener, W. Stöcker, M. Otte, E. Horbach, Doris Kaiser, and P. C. Scriba

Autoantibodies against a component of pancreatic juice (PAb) occur in 39% of patients with Crohn's disease (CD), as determined by indirect immunofluorescence (IIF; 1). In the

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present study double immunodiffusion (DID) testing was used as an alternative method for the detection of PAb, and results were compared to those of IIF. Serum samples from 70 patients with CD were tested, 35 of which were PAb-positive in IIF, the other 35 were PAb-negative. Furthermore, 35 sera from patients with different diagnoses were used which were PAb-negative in IIF. DID was performed with 1 % agarose gels in 0.1 M Tris buffer, pH 8.0, containing 2.5 % polyethyleneglycol 6000. Monoclonal antibody HL-1 to PAg was used as a positive control. PAg was enriched from homogenized human pancreas by size exclusion chromatography and ammonium sulphate precipitation. Gels were incubated for 7 days at 4 °C, dried and stained with Serva blue R. Of 35 sera positive in IIF, 30 showed clear precipitation lines. In 2 other cases precipitation was only weak. Three IIF-positive samples did not react in DID. All sera which were negative in IIF were negative in DID. Reactions of identity were seen with most positive sera, but 5 precipitation lines crossed over, indicating that autoantibodies to exocrine pancreas may react with different epitopes or even different components of the crude antigen preparation used. The epitopes recognized by HL-1 seemed to be different from those reacting with PAb of serum samples. Despite long incubation times, DID is a convenient, easy to handle complementary method for the detection of autoantibodies against exocrine pancreas which enables the diagnosis of many cases of CD in laboratories where IIF testing is not established.

1. Stöcker, W. et al. 1987. Scand. J. Gastroenterol. 22 Suppl. 139: 41–52.

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J.11 Production and immunochemical characterization of islet cell-reactive monoclonal autoantibodies by fusion of spleen cells from diabetic BB rats

M. Schlosser, S. Witt, B. Ziegler, H. Kenk, I. Klöting, and M. Ziegler

The diabetic syndrome of the BB rat shows many homologies with that of human insulin-dependent diabetes and evidence exists that the onset of the disease depends on cellular immune effector mechanisms associated with circulating beta cell-reactive autoantibodies. Evidence for an altered humoral immune response includes the presence of circulating islet cell surface autoantibodies (ICSA) and antibodies against lymphocytes. The aim of this study was to produce monoclonal islet cell-reactive autoantibody (mc-ICRA)-secreting hybridomas and to examine the reactivity with 3 different target preparations for primary screening by ELISA. For this, dried permeable rat insulinoma (RIN) cells, viable RIN cells or homogenized rat pancreatic islets on multiwell microtest plates were used. Two out of ten stable mc-ICRA of IgM class from one fusion were reactive with the surface of RIN cells only and mediate complement dependent cytotoxicity (CAMC) on normal islet cells but not on spleen cells. The other 7 clones were reactive with cytoplasmic antigens of both islet and spleen cells without mediating CAMC. The results suggest that the known lympho- and islet-cytotoxicity in BB rats is caused by different types of antibodies and not only by cross reactivity.

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J.12 The curative effect of 15-deoxyspergualin on the development of SLE-like autoimmune disease in MRL/lpr mice

H. U. Schorlemmer[1], R. R. Bartlett[2], and F. R. Seiler[1]

The polyamine compound 15-deoxyspergualin (15-DOS) has been shown to be a potent suppressor of the monocyte/macrophage system inhibiting several functions like generation of oxygen radicals, secretion of lysosomal enzymes, expression of MHC class I and class II antigens, and production of IL-1. The mode of action on the immune system is clearly different from other immunosuppressants used in clinics, but due to this immunopharmacological profile 15-DOS is suitable for application in autoimmune disorders. Here we examined the prophylactic and therapeutic efficacy of 15-DOS on the development of autoimmune disease in MRL/lpr mice. These autoimmune mice spontaneously develop a systemic lupus erythematosus (SLE)-like disease with clinical and serological characteristics that mimic not only human SLE but other autoimmune disorders, such as rheumatoid arthritis (RA). The disorder of MRL/lpr mice is characterized by excessive T-lymphocyte proliferation and development of autoantibodies directed against double-stranded DNA (dsDNA) and type II collagen. These mice also have circulating rheumatoid factor (RF) and develop histological changes in their joints characterized by pannus formation, cartilage and bone erosions. Treating these mice with 15-DOS resulted in a decrease in the amount of autoantibodies and inhibited proteinuria of the developing glomerulonephritis. 15-DOS treatment also resulted in an improved survival rate of these mice and, at the same time the percentage of animals with swollen lymph nodes was lowered and the development of splenomegaly was inhibited. In the established disease 15-DOS could inhibit the proteinuria and returned urine-protein values and renal function (serum urea and creatinine) to normal levels. Even circulating dsDNA autoantibodies and RF were reduced and the increase in paw volume (signs of a polyarthritis) was inhibited. These results suggest that 15-DOS might be used as a therapeutic agent for human SLE and RA to combat such disorders.

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J.13 Inhibition of various experimental autoimmune diseases by the anti-rat-TCR monoclonal antibody R73

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A murine monoclonal antibody (mAb) BMA 031, directed to the human α/β-T-cell receptor (TCR) is used in clinical trials for prophylaxis and therapy of graft vs. host (GvH) disease because it has a long lasting immunoregulatory effect. Nevertheless, the mechanisms of *in vivo* action of this mAb are not yet fully understood. Due to the fact that BMA 031 is strictly human α/β-TCR specific, and that mAbs directed to TCRs of other species (rats and mice) exist, we compared the *in vivo* effects of the anti-rat-α/β-TCR mAb R73 (generated by T. Hünig) with BMA 031. This rat mAb has been investigated as to its disease modifying activity

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on adjuvant arthritis (AA), on experimental allergic encephalomyelitis (EAE) and a local GvH reaction (popliteal lymph nodes, PLN) in Lewis or Brown-Norway rats. The Ab-R73 was able to prevent the onset of the adjuvant disease, provided the therapy was started within the first 12 days after its induction. If therapy started after the establishment of AA, the antibody was still able to reduce the degree of chronic inflammation and arrest its progress. Intravenous mAb-R73 application also reduced the signs of EAE and prevented mortality. This was even seen when the substance was given after the outbreak of the clinical symptoms or when the $F(ab)_2$ fragment of this anti-rat-α/β-TCR mAb was used. In the model of local GvH reaction the mAb-R73 also acted therapeutically and lowered the PLN weights reflecting an immunosuppressive activity. With the anti-rat-TCR mAb-R73 we could show that in analogy to clinical short term application of BMA 031 in GvH disease a strong immunoregulatory capacity of mAb-R73 is shown in rat autoimmune models. The analogy in model systems makes us confident that the results of therapeutic mAb-protocols in animal models can be directly transferred to protocols for clinical application of BMA 031.

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J.14 Autoantibodies against cerebral gray matter in patients with insulin dependant diabetes mellitus

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Stiff man syndrome (SMS), a rare disease of the central nervous system, is often associated with endocrine disorders, e.g. with insulin-dependent diabetes mellitus (IDDM; 1). SMS-specific autoantibodies were identified. They are directed against gray matter of the brain (GMAb) and can be neutralized with glutamic acid decarboxylase. We observed a 50 year old male patient with both, SMS and IDDM. His serum contained GMAb and stained all regions of the CNS, in which gray matter was represented. The serum exhibited also islet cell antibodies (ICAb), but did not react with 22 other extracerebral human tissues, as analyzed by indirect immunofluorescence. Surprisingly, sera of 20 patients with IDDM, positive for ICAb, reacted in 5 cases with cerebral gray matter, too. In these 5 patients, islet cell as well as gray matter fluorescence could be abolished by a preincubation of the sera with homogenized human *gyrus praecentralis*. ICAb of the other 15 sera could not be neutralized. On the other hand, 20 IDDM-patients negative for ICAb and 100 healthy blood donors did not exhibit GMAbs in the serum. Thus, in some patients with IDDM autoimmune reactions may take place against cerebral gray matter. The clinical association between IDDM and SMS is paralleled by serological phenomena. Furthermore, in IDDM, ICAb with different antigen specifities can be observed. The prevalence of SMS in IDDM should be reevaluated.

1. Howard, F. M. jr. 1963. Mayo Clin. Proc. 38: 421–427.
2. Solimena, M., F. Folli, S. Denis-Donini et al. 1988. N. Engl. J. Med. 318: 1012–1020.

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J.15 IL-6 in adjuvant arthritis of rats and the influence of antirheumatic drugs

P. Theisen-Popp, H. Pape, and R. Müller-Peddinghaus

Adjuvant arthritis of rats (AA) as a T cell-mediated autoimmune disorder is one of the most important experimental models of rheumatoid arthritis (RA). Interleukin-6 (IL-6) is a multifunctional cytokine produced by lymphoid and non-lymphoid cells. Recently the role of IL-6 in autoimmune diseases has been discussed. In RA IL-6 is increased in serum and synovial fluid and it is produced by synoviocytes and chondrocytes. In this study we 1. determined IL-6 in serum and spleen-cell supernatants in the course of AA and 2. we examined the influence of various antirheumatic drugs (indometacin, dexamethason, cyclophosphamide, cyclosporin A) on serum IL-6. *Ad 1:* In the serum of naive rats there was no IL-6 detectable. At day 5 of AA a slight increase could be observed up to 335 pg/ml. A strong increase was determined during the systemic phase of the arthritis with levels up to 5.3 ng/ml at day 12 reaching maximum levels at day 18 (7 ng/ml). Spleen cells of day 12 rats showed the highest production of IL-6 after stimulation with the AA inducing antigen *Mycobacterium tuberculosis* compared to LPS or Con A-stimulated cells. Unstimulated cells showed marginal IL-6 production only. *Ad 2:* From the antirheumatics mentioned above only dexamethasone and cyclosporin A completely inhibited IL-6 production *in vivo* during the whole experiment. Cyclophosphamide totally suppressed the cytokine production at day 12 and to 90% at day 18. Indometacin only partially inhibited IL-6 production. – We conclude that IL-6 is a relevant mediator in chronic inflammation correlating extremely well with disease activity. Immunosuppressive drugs were most efficient to inhibit IL-6 production *in vivo*.

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J.16 Influence of several cytokines on anti-DNA autoantibody secretion by human B cells derived from healthy persons and SLE patients

H.-D. Volk, Ch. Liebenthal, K. Adrian, F. Hiepe, S. Jahn, U. Serfling, and T. Diamantstein

MNC derived from patients with active SLE disease are characterized by a spontaneous hypersecretion of polyclonal IgM/IgG as well as anti-DNA-IgM/IgG *in vitro*. Purified B cells did not show this activity suggesting the dependence on signals by T cells and/or monocytes. Therefore, we asked whether certain cytokines are involved in the regulation of Ig secretion. IL-1, IL-6, TNF-α, IL-2, IL-3, and IL-4 were able to induce a polyclonal IgM as well as anti-DNA-IgM secretion by B cells derived from both SLE patients and healthy volunteers. However, the levels of IgM produced by cytokine-activated B cells derived from SLE patients were higher. Depletion of $CD5^+$ B cells reduced the cytokine-mediated effects. Furthermore, we fused B cells with a heteromyeloma and analyzed the frequency of IgM producing B-cell clones recognizing autoantigens (including anti-DNA). The frequency was 10 times higher in SLE patients than in healthy people. In contrast, polyclonal IgG as well as anti-DNA IgG secretion was observed only in B-cell cultures derived from SLE patients in the presence of IL-

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2 or IL-3. In summary, we have two B-cell subsets: One B cell subset (mostly CD 5$^+$) is sensitive to several cytokines and produces multireactive IgM. This population is also present in healthy people but in lower frequency and activity. A second B-cell subset is able to produce anti-DNA IgG and is only detectable in SLE. The relation between these both subsets (switch/somatic mutation or distinct lineages) will be analyzed at the clonal level.

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J.17 Characterization of human monoclonal anti-DNA antibodies from patients with SLE

T. W. Winkler, M. Herrmann, H. Fehr, F. Krapf, and J. R. Kalden

For the analysis of the molecular mechanisms for autoantibody production in the human systemic lupus erythematosus we focussed our attention on the pathogenic autoreactive B cells derived from the patients. To study the binding profiles and immunoglobulin variable region gene usage of human anti-DNA antibodies we recently established a collection of human anti-DNA hybridomas from SLE patients. For the first time we succeeded in the establishment of up to now six hybridomas of the IgG isotype. These anti-dsDNA antibodies behave like the anti-dsDNA antibodies found with high selectivity in SLE sera in the ELISA, the *Crithidia luciliae* assay and the Farr assay. By competition experiments we could demonstrate a striking specificity of the monoclonal IgG anti-DNA antibodies for certain DNA structures. Nucleic acids derived from the plasma of SLE patients were able to compete the binding to dsDNA very efficiently (50 to 100 fold lower concentrations of this nucleic acids were able to inhibit the binding to the same extent as other synthetic or natural nucleic acids). By separation on agarose gels and subsequent blotting of the plasma nucleic acids we were able to show that the monoclonal antibodies reacted mainly with a fraction comigrating with 1–2 kb linear dsDNA on the gels. These data suggest a nucleic acid antigen circulating in SLE plasma. To analyse whether an antigen-driven selection of anti-DNA reactive B cells is involved we currently sequence the variable gene regions of the heavy and light chains of the IgM and IgG monoclonal anti-DNA antibodies derived from individual patients. For this a specially adapted PCR protocol is used.

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Central Institute of Diabetes «Gerhardt Katsch», Karlsburg, GDR

J.18 Discrepancy of *in vitro* and *in vivo* cytotoxicity of monoclonal and polyclonal islet-cell surface antibodies

S. Witt, B. Ziegler, M. Schlosser, B. Hellmke, and M. Ziegler

Islet cell reactive antibodies (ICRA) binding to cytoplasmic (ICA) and/or cell surface determinants (ICSA) of pancreatic islet cells are detectable in sera of newly diagnosed type 1 diabetics. Although their islet cell toxicity *in vitro* had been detected their role in β-cell destruction is still unclear. By cell fusion we generated hybridomas secreting anti-islet cell monoclonal antibodies (mc-ICSA) from splenocytes of BALB/c mice immunized with isolated

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rat islets, rat insulinoma cells (RIN-5AH) or by β-cell destruction by subdiabetogenic doses of streptozotocin. By immunization of 10 NEDH-rats we received polyclonal (pc) ICRA. Using ELISA and indirect immunofluorescent staining all mc-ICRA and pc-ICRA were shown to bind to antigens at RIN cells and normal rat islet cells. The ICSA also mediated complement dependent cytotoxicity (CMC) *in vitro* but not any of 10 mc-ICSA did induce a hyperglycaemia or reduction of pancreatic insulin content during growth as ascites tumor tested in 43 mice and 10 rats after hyperimmunization with RIN cells in comparison to control animals. Furthermore, four mc-ICSA of IgG class mediating CMC and ADCC *in vitro* were injected daily for 14 days into BALB/c mice with partially reduced β-cell mass. However, all mice remained normoglycaemic. Our data suggest that humoral anti-β-cell toxicity measured *in vitro* does not reflect a cytotoxic potential sufficient for β-cell destruction *in vivo*. Probably ICRA in sera of diabetics are induced against damaged β-cells as a secondary effect but are nevertheless useful as a marker for beta cell destruction.

Institute of Radiology and Pathophysiology, German Cancer Research Center, Heidelberg, FRG

J.19 Idiotypic profile of natural autoantibodies in newborn and young adult BALB/c mice

MARGOT ZÖLLER

In autoimmune disease autoantibodies are frequently characterized by defined idiotypes (ID). In order to explore whether natural autoantibodies may display similar features, the ID profiles of monoclonal antibodies (mAb) binding to one or more of 8 autoantigens were evaluated by their reactivity with a panel of alkaline phosphatase-coupled detector mAb derived from the same fusions. Attention was given to the question whether differences exist between monoclonal autoantibodies derived from spleen cells (SC) or thymocytes (TC) and whether their ID profile may change during postnatal development. In comparison to mAb which did not react with anyone of the 8 autoantigens, natural autoantibodies in the newborn were characterized by the expression of a restricted pattern of ID. This became further narrowed during postnatal development, some ID being significantly overrepresented on SC-derived mAb from young adults. A clear linkage between certain ID and antigen specificities could be demonstrated with TC-derived mAb, which reacted preferentially with only one of the autoantigens. These features support the hypothesis of an antigen-/T cell-driven expansion and maturation of natural autoantibodies.

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J.20 Autoreactive B cells in thymus and spleen of neonatal and young adult BALB/c mice: influence of prenatal tolerization

MARGOT ZÖLLER and MARTIN ACHTNICH

To pursue the question whether natural autoantibodies represent a random collection of immunoglobulins with germline sequences or whether naturally activated B cells may be part

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of an inherently regulated primary defense system, patterns of autoreactivity of hybridoma collections derived from the thymus and the spleen of 6 and 28 days old prenatally untreated and tolerized (TNBS-treated) mice were compared. Monoclonal antibodies (mAb) were tested for their reactivity with the autoantigens thyroglobulin (TG), myoglobulin (MY), actin (AC), cytochrome (CY), collagen (CO), transferrin (TF), single stranded and double stranded (ss/ds) DNA and bromelain-treated mouse red blood cells (BrMRBC). More than 10 % of spleen cell-derived mAb of 6 and 28 day old untreated mice did bind to AC, ssDNA, MY and TG, reactivity with MY, TG and BrMRBC increasing with age. Thymocyte-derived mAb contained autoreactive mAb, too, but degeneracy was less pronounced. In prenatally tolerized mice a significantly higher number of mAb was autoreactive and degeneracy was more pronounced. Many spleen cell-derived mAb did bind to AC and TG, while the majority of thymocyte-derived mAb reacted with dsDNA, the preimmune repertoire being known to include mainly ssDNA-specific B cells. The differences in autoreactivity between mAb from prenatally untreated and TNBS-treated mice as well as age- and organ-related variations support the interpretation that part of the repertoire of naturally activated B cells is not random, but is influenced by and responding to the available panel of self antigens.

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Workshop K
Cellular Aspects of Autoimmunity

Physiological Chemistry and I. Department of Internal Medicine, University of Mainz, FRG

K.1 Characterization of the autoantigen La (SS-B) as a nucleic acid dependent ATPase with denaturing properties: Appearance on the cell surface

M. Bachmann, S. Chang, W.-J. Mayet, K.-H. Meyer zum Buschenfelde, and W. E. G. Müller

Sera of patients with autoimmune diseases frequently contain autoantibodies recognizing the so called La antigen. We developed monoclonal antibodies (mAbs) against La protein (1). With these mAbs we showed that La protein is an enzyme, a nucleic acid dependent ATPase/dATPase with denaturing properties (2). It might function as a transcription/termination factor of RNA polymerase III denaturing the nascent transcripts from the DNA template (2). From our immunolocalization studies confirmed now with microinjection experiments it is evident that La protein is not restricted to the nucleus but shuttles between nucleus and cytoplasm (3). Both, after virus infections and stress situations, like UV irradiation or starving and refeeding, La protein appears on the cell surface (4). Here La protein colocalized with EGF receptors and MHC antigens. After a herpes virus infection La protein was found in large protrusions on the cell surface. Besides La protein these protrusions contained cellular actin, fibronectin and virions.

Bachmann et al.: (1) Proc. Natl. Acad. Sci. USA **83**: 7770 (1986); (2) Cell **60**: 85 (1990); (3) Mol. Cell. Biochem. **85**: 103 (1989); (4) Mol. Biol. Rep. **12**: 49 (1990).

Diabetes Research Institute, Heinrich Heine University Düsseldorf, FRG

K.2 Macrophage-mediated cytotoxicity against pancreatic islet cells is suppressed by calcium dobesilat

H.-H. Brenner, V. Burkart, T. Koike, and H. Kolb

Human type 1 diabetes is an autoimmune disease which is characterized by a continuous loss of insulin-producing pancreatic beta cells. In animal models of this disease it was shown that activated macrophages may play an important role in this process. Recently we have demonstrated that activated macrophages are able to lyse syngeneic ^{3}H-leucine labelled pancreatic islet cells after 15 h coculture in an *in vitro* cytotoxic assay. In this test system islet

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cells were killed up to 88.9% at a target/effector (T/E) ratio of 1:40. The macrophage-sensitive mouse mastocytoma cells P815 were killed to 89.0% and the tumor necrosis factor (TNF)-sensitive mouse fibroblast cells L929 up to 51.5%. The addition of calcium dobesilat (Doxium, Laboratoires OM), a well-established drug in the treatment of diabetic vascular complications, into the coculture suppressed lysis of islet cells, P815 cells and L929 cells (concentration for 50% suppression 0.2–1 mM). Other experiments showed, that calcium dobesilat suppressed zymosan-induced release of reactive oxygen intermediates of activated macrophages from a peak activity of 19.6×10^6 cpm (control) to 3.1×10^6 cpm at a concentration of 1 mM calcium dobesilat. Calcium dobesilat not only suppressed macrophage activity but also protected L929 cells from the toxic effects of recombinant TNF-α (50% protection at 0.9 mM). Our results suggest that calcium dobesilat may protect islet cells from macrophage mediated lysis by counteracting the effector mechanisms of activated macrophages.

University Clinics in Hannover, Düsseldorf, Erlangen, Freiburg, München, Bonn, Mainz, Bielefeld, Ancona; SRK Bern; NRC Amsterdam

K.3 Results of the SLE multicenter study – associations of immunogenetic factors in 400 patients and 130 families

K. Hartung, J. R. Kalden, H. J. Lakomek, H. H. Peter, H. Deicher, and the members of the SLE-Study Group*

The aim of this study is the evaluation of genetic factors in SLE and their impact on autoantibody formation and the clinical spectrum of this disease. Present results obtained from over 300 SLE-patients show a significant over-representation of HLA-DR3, DR2, and B8. As affirmed in the family study, two haplotypes – B7DR2 *and* B8DR3 – are increased in SLE. C4AQ0 alleles are also increased in SLE, mainly due to C4A deletions in the B8DR3 haplotype. Preliminary data do not indicate an increase of C4AQ0 alleles without deletions. The frequency of C4BQ0 frequencies does not differ from controls. The analysis of the role of MHC alleles in autoantibody formation showed that even after exclusion of all potential carriers of the B8DR3 haplotype, Ro and La antibodies are still strongly associated with DR3, implying that DR3 (and not the B8DR3 haplotype) is associated with these autoantibodies. Other autoantibodies tested, including cardiolipin antibodies, showed only marginal MHC-associations. Contrary to previous reports, no significant associations with immunoglobulin allotypes were found in 321 SLE patients. To definitely prove whether C4A deletions or the association with DR antigens are the decisive factor for the MHC-association of SLE, haplotypes of patients and family members are presently analyzed in 130 families, and genomic DNA samples are investigated by RFLP.

* E. Albert, M. Baur, R. Coldewey, A. Corvetta, J. Frey, F. Krapf, B. Lang, G. G. de Lange, R. Müller, E. Röther, D. Schendel, P. Schneider, S. Seuchter, Ch. Specker, P. Späth, S. Stannat

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[1]I. Department of Internal Medicine, Johannes-Gutenberg-Universität, 6500 Mainz, FRG, and [2]Laboratory of Bacteriology, National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands

K.4 A *Yersinia*-reactive synovial T-cell clone which recognizes the mycobacterial and the human 65 kD heat-shock protein (hsp) in *Yersinia* arthritis

E. Hermann[1], A. W. Lohse[1], W. J. Mayet[1], P. Probst[1], R. van der Zee[2], J. D. A. van Embden[2], K.-H. Meyer zum Buschenfelde[1], and B. Fleischer[1]

Heat-shock proteins (hsp) are induced in bacteria and in eukaryotes under various conditions of stress. Since bacterial hsp of the 60 kD hsp family have been highly conserved during evolution and concurrently seem to be markedly immunogenic it is conceivable that an antibacterial immune response during inflammation might induce an autoimmune reaction to a cross-reacting self epitope. In the present study we have examined synovial fluid (SF) T lymphocytes of a patient with *Yersinia* arthritis, a reactive arthritis known to be initiated by an extraarticular bacterial infection and to be perpetuated by immune reactions in predisposed patients. Bulk SF T-cell proliferation assays of this patient showed responses to heat-killed *Yersinia enterocolitica, Salmonella typhimurium* as well as to the mycobacterial and the human 65 kD hsp. SF derived T-cell lines were raised by repeated stimulation with *Yersinia* antigens and autologous irradiated feeder cells and were subcloned by the limiting dilution technique. We obtained a $CD4^+$ $CD8^-$ $\alpha\beta^+$ $V\beta5^-$ $V\beta8^-$ T-cell clone (JP1.2) which proliferated in response to *Yersinia* (stimulation index (SI) = 86), *Salmonella* (SI = 35), the mycobacterial 65 kD hsp (SI = 75) and the human 65 kD hsp (SI = 67). Other *Yersinia*-reactive clones from the same cloning experiment were specific for *Yersinia* alone or cross-reacting with *Salmonella*, but not with the 65 kD hsp. JP1.2, but none of the control clones also proliferated in response to autologous stressed antigen presenting cells. These results demonstrate at a clonal level a cross-reacting autoimmune T-cell response induced by a natural bacterial infection.

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K.5 Immunomodulatory treatment of rheumatoid arthritis with an anti-CD4(anti-helper T cell) monoclonal antibody

G. Horneff, G. R. Burmester, F. Emmrich, and J. R. Kalden

Ten patients with severe untractable rheumatoid arthritis were treated with the monoclonal anti-CD4 antibody 16H5 in a dosage of 0.3 mg/kg body weight on 7 subsequent days. There was a drastic depletion of $CD4^+$ cells down to a minimum of 25 cells/μl 1 hour after infusion with a certain recovery of the number of $CD4^+$ cells 24 hours after injection. The full treatment cycle, however, resulted in a persistent reduction of $CD4^+$ cells with an inversed CD4/CD8 ratio that usually persisted 3–4 weeks. T helper cells remaining after the infusion of the antibody showed a modulation of the CD4 antigen with a strikingly decreased antigen density down to 20%. Cell lysis was suggested by the presence of complement split products

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on the cell surface and highly elevated serum levels of soluble CD4 antigens. T cell reactivity was markedly reduced immediately after infusion of the antibody, but was unexpectedly enhanced at the time of cell recovery. Parallel studies showed a significant reduction of the erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor and total immunoglobulin values. Clinical benefits were a significant reduction of the Ritchie index, increase of grip strength, decrease of morning stiffness, and reduced numbers of swollen joints. Adverse effects consisted of skin urticaria in two patients and chills with fever presumably due to the release of TNF and IL-2 in another two patients. Only low levels of human anti-mouse immunoglobulin antibodies developed in the patients treated. Therefore, it was possible to repeat the treatment cycles with even a better efficacy in four patients. These data suggest that treatment with monoclonal antibodies against the CD4 antigen present on helper T cells leads to immunomodulation resulting in clinical benefits without severe adverse effects.

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K.6 Specificity analysis of T cell lines derived from synovial fluid lymphocytes of rheumatoid arthritis patients

Jutta Jooss-Rüdiger, Ulrike Isserstedt, and Inga Melchers

T cell clones from the synovial fluid of rheumatoid arthritis (RA) patients were established using 2 different strategies: a) cloning *in vivo* preactivated T lymphocytes by expansion in interleukin-2 (IL-2) containing medium in the presence of autologous feeder cells; b) cloning without selection under optimal conditions (allogeneic feeder cells plus PHA). Up to now we have tested more than 250 T cell clones and lines of four HLA-DR4 positive RA patients, by stimulating them with 1) autologous peripheral blood lymphocytes, 2) autologous EBV-transformed B cells, 3) DR4 transfected cells. As antigens we used various collagens, cartilage extract (kindly provided by K. von der Mark, Erlangen), *Mycobacterium tuberculosis (M. tub.)* and 65 kD heat shock protein (hsp65) of *M. tub.* (kindly provided by S. H. E. Kaufmann, Ulm). Interestingly, about one third of the T cell lines tested showed a high degree of autoreactivity after incubation with the autologous EBV lines and/or after stimulation with autologous peripheral blood lymphocytes. In addition, we detected clones which proliferated after stimulation with *M. tub.* or with a cartilage extract. No stimulation was observed with collagen I, II, IV or V, or with hsp65.

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K.7 T-cell clonality in joints of rheumatoid arthritis patients

R. A. Kroczek[1], B. Hennerkes[1], H. Menninger[2], J. Zacher[3], and F. Emmrich[1]

The role of T cells at the site of inflammation in rheumatoid arthritis (RA) is ill understood. If immunodominant antigens are driving the disease process one can anticipate a clonal T-cell response. The goal of our study was to prove or disprove this hypothesis by using the

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rearrangement patterns of the T-cell receptor (TCR) β- und γ-chains as clonal markers. T-cells from inflamed synovial tissue were expanded *in vitro* under primary limiting dilution conditions with PHA^+ $IL\text{-}2^+$ conditioned medium. The aim was to generate panels of T-cell clones representative of the original T-cell populations *in vivo*. Panels of 31, 67, and 96 T-cell clones were grown from individual patients with a cloning efficiency of 1:3 to 1:5 and examined by restriction mapping of the TCR-DNA using Southern blotting (EcoRI, HindIII, BamHI-digests, Cβ1- and Jγ1-probes). Our analysis did not reveal T-cell clonality within the 3 «representative» panels. In a second approach aimed at selectively cloning out pre-activated T cells from synovial fluid, 4 panels totalling 108 T cell clones were generated by limiting dilution and analyzed by restriction mapping of the TCR. In 3 out of the 4 panels we found clear evidence of T-cell clonality (17 out of 38, 5 out of 28, 2 and 3 identical out of 25). In the second case we were also able to examine 50 T-cell clones generated from the patients' peripheral blood (PB) using identical culture conditions: no clonality was found there. Using PB of healthy donors for controls, we found 2 out of 28, and none out of 38 T-cell clones to be identical. At present we are molecularly cloning and sequencing some of the clonal TCRs. This will enable us to generate TCR-V-region specific cDNA probes and monoclonal antibodies. With these probes at hand we intend to examine the usage and location of T cells bearing particular V-regions in patients with RA.

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K.8 Assembly of the Ro/SS-A antigen with the intermediate filament system

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Skin lesions, especially at areas exposed to sunlight, prove to be a major form of manifestation of diseases related to Ro/SS-A antibodies. In 1986 we described a monoclonal antibody (mAb) against the Ro/SS-A antigen (1). Performing immunofluorescence microscopy on HEp2-cells, one striking property of this mAb was the reaction with cytokeratin filaments (1). Though some Ro/SS-A positive sera show a similar staining pattern (2) it remained unclear whether the Ro/SS-A antigen shares a common epitope with an intermediate filament protein (cross-reaction of the antibody) or whether the Ro/SS-A antigen really associates with the intermediate filament. Hela-cells were extracted with Triton-100 and fixed with 3.7% paraformaldehyde. After reaction with the Ro/SS-A mAb, cells were incubated with a FITC- or 8 nm colloid-gold conjugated antibody. Cells were analyzed by immunofluorescence microscopy or electronmicroscopy, respectively. At the level of electronmicroscopy it became evident that the Ro/SS-A mAb does not directly label the intermediate filament system but stains globular structures associated with intermediate filaments. These globular structures presumably represent the Ro/SS-A RNPs. Some of these RNPs were also labelled with antibodies to La/SS-B protein and novel mAbs against additional components in these RNPs. Some hypotheses on the direct pathogenicity of the Ro/SS-A antibody already exist. Association of the Ro/SS-A antigen with cytoskeletal components as described here could be a strong argument for this assumption concerning destruction of basal layers of human epidermis in subacute cutaneous lupus erythematosus (SCLE).

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2. Mayet, W.-J. et al. Eur. J. Clin. Invest. 18: 465–471 (1988).

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